



AD-A203 827

Institute Report No. 322

**Mutagenic Potential of
2-(E)-Hydroxyiminomethyl-1-methoxymethyl-3-
methylimidazolium Chloride in the Ames
Salmonella/Mammalian Microsome Mutagenicity Test**

*Steven K. Sano, BA, SGT, USA
and
Don W. Korte, Jr., PhD, MAJ, MSC*

GENETIC TOXICOLOGY BRANCH
DIVISION OF TOXICOLOGY

DTIC
ELECTE
S JAN 25 1989 D
a H

November 1988

Toxicology Series: 122

DECLASSIFICATION STATEMENT A

Approved for public release;
Distribution Unlimited

LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

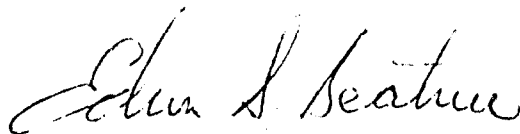
Mutagenic Potential of 2-(E)-Hydroxyiminomethyl-1-methoxymethyl-3-methylimidazolium Chloride in the Ames *Salmonella*/Mammalian Microsome Mutagenicity Test (Toxicology Series 122)--Sano and Korte

This document has been approved for public release and sale; its distribution is unlimited.

Destroy this report when it is no longer needed. Do not return to the originator.

Citation of trade names in this report does not constitute an official endorsement or approval of the use of such items.

This material has been reviewed by Letterman Army Institute of Research and there is no objection to its presentation and/or publication. The opinions or assertions contained herein are the private views of the author(s) and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense. (AR 360-5)



**Edwin S. Beatrice
COL, MC
Commanding**

5 Dec 88

(date)

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION UNCLASSIFIED		1b. RESTRICTIVE MARKINGS	
2a. SECURITY CLASSIFICATION AUTHORITY		3. DISTRIBUTION / AVAILABILITY OF REPORT APPROVED FOR PUBLIC RELEASE; DISTRIBUTION IS UNLIMITED.	
2b. DECLASSIFICATION / DOWNGRADING SCHEDULE		5. MONITORING ORGANIZATION REPORT NUMBER(S)	
4. PERFORMING ORGANIZATION REPORT NUMBER(S) Institute Report No.: 322			
6a. NAME OF PERFORMING ORGANIZATION Genetic Toxicology Branch Division of Toxicology	6b. OFFICE SYMBOL (If applicable) SGRD-ULE-T	7a. NAME OF MONITORING ORGANIZATION Walter Reed Army Institute of Research	
6c. ADDRESS (City, State, and ZIP Code) Letterman Army Institute of Research Presidio of San Francisco, CA 94129-6800		7b. ADDRESS (City, State, and ZIP Code) Washington, DC, 20307-5100	
8a. NAME OF FUNDING / SPONSORING ORGANIZATION US Army Medical Research & Development Command	8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER	
8c. ADDRESS (City, State, and ZIP Code) Fort Detrick Frederick, Maryland 21701-5012		10. SOURCE OF FUNDING NUMBERS	
		PROGRAM ELEMENT NO. 62734	PROJECT NO. A875
		TASK NO. BC	WORK UNIT ACCESSION NO. DA0H0366
11. TITLE (Include Security Classification) (U) Mutagenic Potential of 2-(E)-Hydroxy- iminomethyl-1-methoxymethyl-3-methylimidazolium Chloride in the Ames Salmonella/Mammalian Microsome Mutagenicity Test			
12. PERSONAL AUTHOR(S) SK Sano and DW Korte, Jr.			
13a. TYPE OF REPORT Institute	13b. TIME COVERED FROM 25FEB85 to 22MAR85	14. DATE OF REPORT (Year, Month, Day) November 1988	15. PAGE COUNT 20
16. SUPPLEMENTARY NOTATION Toxicology Series No. 122			
17. COSATI CODES		18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)	
FIELD	GROUP	SUB-GROUP	
		Mutagenicity, Genetic Toxicology, Ames Test, 2-(E)-Hydroxyiminomethyl-1-methoxymethyl-3- methylimidazolium chloride, Oxime, imidazole s. (Mg)	
19. ABSTRACT (Continue on reverse if necessary and identify by block number) The mutagenic potential of 2-(E)-HYDROXYIMINOMETHYL-1-METHOXYMETHYL-3-METHYLIMIDAZOLIUM CHLORIDE was assessed by using the Ames Salmonella/Mammalian Microsome Mutagenicity Test. Tester strains TA98, TA100, TA1535, TA1537, and TA1538 were exposed to doses ranging from 5.0 mg/plate to 0.0016 mg/plate. The test compound was not mutagenic under conditions of this test.			
20. DISTRIBUTION / AVAILABILITY OF ABSTRACT <input checked="" type="checkbox"/> UNCLASSIFIED/UNLIMITED <input type="checkbox"/> SAME AS RPT. <input type="checkbox"/> DTIC USERS		21. ABSTRACT SECURITY CLASSIFICATION UNCLASSIFIED	
22a. NAME OF RESPONSIBLE INDIVIDUAL EDWIN S. BEATRICE, COL, MC		22b. TELEPHONE (Include Area Code) (415) 561-3600	22c. OFFICE SYMBOL SGRD-ULZ

DD Form 1473, JUN 86

Previous editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE

UNCLASSIFIED

ABSTRACT

The mutagenic potential of 2-(E)-HYDROXYIMINOMETHYL-1-METHOXYMETHYL-3-METHYLIMIDAZOLIUM CHLORIDE was assessed by using the Ames *Salmonella*/Mammalian Microsome Mutagenicity Test. Tester strains TA98, TA100, TA1535, TA1537, and TA1538 were exposed to doses ranging from 5.0 mg/plate to 0.0016 mg/plate. The test compound was not mutagenic under conditions of this test.

Key Words: Mutagenicity, Genetic Toxicology, Ames Test, 2-(E)-HYDROXYIMINOMETHYL-1-METHOXYMETHYL-3-METHYLIMIDAZOLIUM CHLORIDE, Oxime



Accession For	
NTIS GRA&I	<input checked="" type="checkbox"/>
DTIC TAB	<input type="checkbox"/>
Unannounced	<input type="checkbox"/>
Justification	
By	
Distribution/	
Availability Codes	
Dist	Avail and/or Special
A-1	

PREFACE

TYPE REPORT: Ames Test GLP Study Report

TESTING FACILITY:

US Army Medical Research and Development Command
Letterman Army Institute of Research
Presidio of San Francisco, CA 94129-6800

SPONSOR:

US Army Medical Research and Development Command
Walter Reed Army Institute of Research
Washington, D.C. 20307-5100
Project Officer: H.A. Musallam

PROJECT/WORK UNIT/APC: 3M162734A875/308/TLEO

GLP STUDY NUMBER: 85007

STUDY DIRECTOR: MAJ Don W. Korte, Jr., PhD, MSC

PRINCIPAL INVESTIGATOR: Steven K. Sano, BA, SGT, USA

REPORT AND DATA MANAGEMENT:

A copy of the final report, study protocol, retired SOPs, stability and purity data on the test compound, and an aliquot of the test compound will be retained in the LAIR Archives.

TEST SUBSTANCE: 2-(E)-HYDROXYIMINOMETHYL-1-METHOXYMETHYL-3-METHYLIMIDAZOLIUM CHLORIDE

INCLUSIVE STUDY DATES: 25 February 1985 - 22 March 1985

OBJECTIVE:

The objective of this study was to determine the mutagenic potential of 2-(E)-HYDROXYIMINOMETHYL-1-METHOXYMETHYL-3-METHYLIMIDAZOLIUM CHLORIDE (LAIR Code TP50) by using the Ames *Salmonella*/Mammalian Microsome Mutagenicity Test.

ACKNOWLEDGMENTS

MAJ John W. Harbell, PhD, MSC, and Mr. John Dacey provided scientific guidance and research assistance.

**SIGNATURES OF PRINCIPAL SCIENTISTS INVOLVED IN THE
STUDY**

We, the undersigned, declare that GLP Study 85007 was performed under our supervision, according to the procedures described herein, and that this report is an accurate record of the results obtained.

Don W. Korte Jr. 30 NOV 88
DON W. KORTE JR, PhD / DATE
MAJ, MSC
Study Director

Steven K. Sano 5 MAR 86
STEVEN K. SANO, BA / DATE
SGT, USA
Principal Investigator

Conrad Wheeler 14 July 88
CONRAD WHEELER, PhD / DATE
DAC
Analytical chemist



DEPARTMENT OF THE ARMY

LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129-6800

REPLY TO
ATTENTION OF:

SGRD-ULZ-QA

3 December 1988

MEMORANDUM FOR RECORD

SUBJECT: GLP Statement of Compliance

1. This is to certify that the protocol for GLP Study 85007 was reviewed on 21 February 1985.
2. The institute report entitled "Mutagenic Potential of 2-(E)-Hydroxyiminomethyl-1-methoxymethyl-3-methylimidazolium Chloride in the Ames Salmonella/Mammalian Microsome Mutagenicity Test," Toxicology Series 122, was audited on 14 November 1988.

Carolyn M. Lewis

CAROLYN M. LEWIS, MS
Diplomate, American Board of Toxicology
Chief, Quality Assurance

TABLE OF CONTENTS

Abstract	i
Preface	iii
Acknowledgments	iv
Signatures of Principal Scientists	v
Report of the Quality Assurance Unit	vi
Table of Contents	vii
INTRODUCTION	1
Objective of the Study	1
MATERIALS AND METHODS	1
Test Compound	1
Test Solvent	2
Chemical Preparation	2
Test Strains	2
Test Format	2
Data Interpretation	4
Deviations from the Protocol/SOP	4
Storage of the Raw Data and Final Report	4
RESULTS	5
DISCUSSION	5
CONCLUSION	10
REFERENCES	11
APPENDICES	12
Appendix A: Chemical Data	13
Appendix B: Individual Plate Scores	15
OFFICIAL DISTRIBUTION LIST	20

Mutagenic Potential of 2-(E)-HYDROXYIMINOMETHYL-1-METHOXYMETHYL-3-METHYLIMIDAZOLIUM CHLORIDE in the Ames Salmonella/Mammalian Microsome Mutagenicity Test--Sano and Korte

INTRODUCTION

2-(E)-HYDROXYIMINOMETHYL-1-METHOXYMETHYL-3-METHYLIMIDAZOLIUM CHLORIDE was synthesized for a United States Army Medical Research and Development Command program charged with developing more effective oximes for treatment of nerve agent poisoning. The Ames Test is one of a series of tests in which these compounds will be evaluated to determine their relative potential for further development.

The Ames *Salmonella*/Mammalian Microsome Mutagenicity Test is a short-term screening test that utilizes histidine auxotrophic mutant strains of *Salmonella typhimurium* to detect compounds that are potentially mutagenic in mammals. A mammalian microsomal enzyme system is incorporated in the test to increase sensitivity by simulating *in vivo* metabolic activation of the test compound. The Ames Test is an inexpensive yet highly predictive and reliable test for detecting mutagenic activity and thus carcinogenic potential (1).

Objective of the Study

The objective of this study was to determine the mutagenic potential of 2-(E)-HYDROXYIMINOMETHYL-1-METHOXYMETHYL-3-METHYLIMIDAZOLIUM CHLORIDE (LAIR Code TP50) by using the revised Ames *Salmonella*/Mammalian Microsome Mutagenicity Test.

MATERIALS AND METHODS

Test Compound

Chemical Name: 2-(E)-HYDROXYIMINOMETHYL-1-METHOXYMETHYL-3-METHYLIMIDAZOLIUM CHLORIDE

LAIR Code Number: TP50

Physical State: Colorless prisms

Source: SRI International, Menlo Park, CA

Storage: 2-(E)-HYDROXYIMINOMETHYL-1-METHOXYMETHYL-3-METHYLIMIDAZOLIUM CHLORIDE was received from SRI International, 333 Ravenswood Ave., Menlo Park, CA 94025 and assigned the LAIR Code number TP50. The test compound was stored in a desiccator at 5°C until used.

Chemical Properties/Analysis: Data provided by SRI International characterizing the chemical composition and purity of the test material are presented in Appendix A along with confirmatory analysis of the test material performed by the Division of Toxicology, LAIR (Presidio of San Francisco, CA).

Test Solvent

The positive control chemicals were dissolved in grade I dimethyl sulfoxide (lot 113F-0450) obtained from Sigma Chemical Co. (St. Louis, MO). The test compound was dissolved in sterile deionized water obtained from a Polymetric model 200-3 Water Purifier (Sunnyvale, CA).

Chemical Preparation

On the day of dosing, 300 mg of the test compound was measured into a sterile vial and dissolved in 6 ml of sterile deionized water to achieve a 5% (w/v) solution. Aliquots of this solution were used to dose the test plates.

Test Strains

Salmonella strains TA98, TA100, TA1535, TA1537, and TA1538 obtained directly from Dr. Bruce Ames, University of California, Berkeley, were used. These strains were maintained in our laboratory in liquid nitrogen. Quality control tests were run concurrently with the test substance to establish the validity of their special features and to determine the spontaneous reversion rate. Descriptions of the strains, their genetic markers, and the methods for strain validation are given in the LAIR SOP, OP-STX-1 (2).

Test Format

2-(E)-HYDROXYIMINOMETHYL-1-METHOXYMETHYL-3-METHYLIMIDAZOLIUM CHLORIDE was evaluated for mutagenic potential according to the methods of Ames et al (3). A detailed description of the methodology is given in LAIR SOP, OP-STX-1 (2).

Toxicity Tests:

Toxicity tests were conducted to determine a sublethal concentration of the test substance. This toxicity level was found by using minimal glucose agar (MGA) plates, concentrations of 2-(E)-HYDROXYIMINOMETHYL-1-METHOXYMETHYL-3-METHYLIMIDAZOLIUM CHLORIDE ranging from 1.6×10^{-3} mg/plate to 5 mg/plate, and approximately 10^8 cells of TA100 per plate. Top agar containing trace amounts of histidine and biotin was placed on the plates. Strain verification was confirmed on the bacteria, along with a determination of the spontaneous reversion rate. After incubation, the growth on the plates was observed. Since none of the plates showed a decreased number of macrocolonies (below the spontaneous rate) or an observable reduction in the density of the background lawn, the highest dose selected for the mutagenicity test was 5.0 mg/plate.

Mutagenicity Test:

The test substance was evaluated over a 1000-fold range of concentrations, decreasing from the minimum toxic level (the maximum or limit dose) by a dilution factor of 5, both with and without 0.5 ml of the S-9 microsome fraction. The S-9 (batch R-315) was purchased from Litton Bionetics (Kensington, MD). The optimal titer of this S-9, as determined by Litton Bionetics, was 0.75 mg protein/plate. After all the ingredients were added, the top agar was mixed, then overlaid on MGA plates. These plates contained 2% glucose and Vogel Bonner "E" concentrate (4). Plates were incubated upside down in the dark at 37°C for 48 hours. Plates were prepared in triplicate, and the average revertant counts were recorded. The average number of revertants at each dose level was compared to the average number of spontaneous revertants (negative control). The spontaneous reversion rate (with and without S-9) was monitored by averaging the counts from two determinations run simultaneously with the test compound. The spontaneous reversion rate was determined by inoculating one set of plates before and one set after the test compound plates so that any change in spontaneous reversion rate during the dosing procedure would be detected. This spontaneous reversion rate was also compared with historical values for this laboratory and those cited in Ames et al (3). Sterility and strain verification controls were run concurrently. All reagents, test compounds, and media were checked for sterility by plating samples of each on MGA media and incubating them at 37°C with the test plates. The integrity

of the different *Salmonella* strains used in the assay was verified by the following standard tests:

- Lack of growth (inhibition) in the presence of crystal violet which indicates that the prerequisite alteration of the lipopolysaccharide layer (LP) of the cell wall is present.
- Growth in the presence of ampicillin-impregnated disks which indicates the presence of an ampicillin-resistant R Factor in the TA98 and TA100 strains.
- Lack of growth (inhibition) following exposure to ultraviolet light which indicates the absence of the DNA excision-repair mechanism.

Four known mutagens were tested as positive controls to confirm the responsiveness of the strains to the mutation process. Each strain must be tested with at least one positive control but may be tested with several. These compounds (benzo[a]pyrene, 2-aminofluorene, 2-aminoanthracene, and N-methyl-N'-nitro-N-nitrosoguanidine) were obtained from Sigma Chemical Co. (St. Louis, MO). The test compound and mutagens were handled during this study in accordance with the standards published in NIH Guidelines for the Laboratory Use of Chemical Carcinogens (DHHS Publication No. (NIH) 81-2385, May 1981).

Data Interpretation

According to Brusick (5), a compound is considered mutagenic if a positive dose response (correlated dose response) over three dose concentrations is achieved with at least the highest dose yielding a revertant colony count greater than or equal to twice the spontaneous colony count for the tester strains TA98 and TA100, or three times the spontaneous colony count for strains TA1535, TA1537, and TA1538. A strong correlated dose response in strain TA100 without a doubling of the individual colony count may also be considered positive.

Deviations from the Protocol/SOP

There were no deviations from the protocol or standard operating procedures.

Storage of the Raw Data and Final Report

A copy of the final report, study protocols, raw data, SOPs, and an aliquot of the test compound will be retained in the L-IR Archives.

RESULTS

On 8 March 1985, the toxicity of 2-(E)-HYDROXYIMINOMETHYL-1-METHOXYMETHYL-3-METHYLIMIDAZOLIUM CHLORIDE was determined (Table 1). For this experiment all sterility, strain verification and negative controls were normal (Table 1). Exposure of the tester strain (TA100) to the highest dose showed neither a decrease in the number of macrocolonies nor an observable reduction in the density of the background lawn. Therefore, the highest dose selected for the mutagenicity test was 5.0 mg/plate. Normal results were obtained for all sterility and strain verification tests during the Ames Test performed on 11-14 March 1985 (Table 2). 2-(E)-HYDROXYIMINOMETHYL-1-METHOXYMETHYL-3-METHYLIMIDAZOLIUM CHLORIDE did not induce any appreciable increase in the revertant colony counts relative to those of the negative control cultures (Table 3). A tabular presentation of the raw data is included in Appendix B.

DISCUSSION

Certain test criteria must be satisfied before an Ames Test can be considered a valid assessment of a compound's mutagenic potential. First, the special features of the Ames strains must be verified. These features include demonstration of ampicillin resistance, alterations in the LP layer, and deficiency in DNA excision-repair (except TA102). Second, the *Salmonella* strains must be susceptible to mutation by known mutagens. Third, the optimal concentration of the test compound must be determined by treating TA100 with a broad range of doses and observing the potential toxic effects on formation of macrocolonies and microcolonies. If these tests are performed and expected data are obtained, then the results of an Ames Test can be considered valid.

After validation of bacterial strains and selection of optimal sublethal doses, 2-(E)-HYDROXYIMINOMETHYL-1-METHOXYMETHYL-3-METHYLIMIDAZOLIUM CHLORIDE was evaluated in the Ames Test. Criteria for a positive response include both a correlated dose response over three dose concentrations, and a revertant colony count at least two times (TA98, TA100) or three times (TA1535, TA1537, TA1538) the spontaneous revertant colony count (5). 2-(E)-HYDROXYIMINOMETHYL-1-METHOXYMETHYL-3-METHYLIMIDAZOLIUM CHLORIDE did not induce the requisite dose-response relationship or the increase in revertant colony counts necessary for a positive response. Thus, the results of this test indicate that 2-(E)-HYDROXYIMINOMETHYL-1-METHOXYMETHYL-3-METHYLIMIDAZOLIUM CHLORIDE is not mutagenic when evaluated in the Ames Test.

TABLE 1: TOXICITY LEVEL DETERMINATION FOR TP50

GLP STUDY NUMBER 85007

<u>TOXICITY DETERMINATION REVERTANT PLATE COUNT (TA100)</u>			
<u>CONCENTRATION</u>	<u>MEAN</u>	<u>±1SD</u>	<u>BACKGROUND LAWN*</u>
5.0 mg/plate	108	31.8	NL
1.0 mg/plate	112	12.8	NL
0.2 mg/plate	122	5.9	NL
0.04 mg/plate	121	13.2	NL
0.008 mg/plate	127	14.0	NL
0.0016 mg/plate	122	8.5	NL

STRAIN VERIFICATION FOR TOXICITY DETERMINATION

	<u>TA100*</u>
HISTIDINE REQUIREMENT	NG
AMPICILLIN RESISTANCE	G
UV	NG
CRYSTAL VIOLET SENSITIVITY	NG
STERILITY CONTROL	NG

STERILITY CONTROL FOR TOXICITY DETERMINATION

<u>MATERIAL TESTED</u>	<u>OBSERVATION*</u>
MINIMAL GLUCOSE AGAR PLATES	NG
TOP AGAR	NG
DILUENT WATER	NG
NUTRIENT BROTH	NG
TEST COMPOUND (HIGHEST DOSE)	NG

*NL=Normal Lawn, G=Growth, NG=No Growth

**TABLE 2: STRAIN VERIFICATION AND STERILITY TESTING
FOR THE MUTAGENICITY DETERMINATION OF TP50**

GLP STUDY NUMBER 85007

STRAIN VERIFICATION					
STRAIN	OBSERVATIONS*				
	HISTIDINE REQUIREMENT	AMPICILLIN RESISTANCE	UV REPAIR	CRYSTAL VIOLET	STERILITY CONTROL
TA98	NG	G	NG	NG	NG
TA100	NG	G	NG	NG	NG
TA1535	NG	NG	NG	NG	NG
TA1537	NG	NG	NG	NG	NG
TA1538	NG	NG	NG	NG	NG

STERILITY CONTROL FOR MUTAGENICITY DETERMINATION

<u>MATERIAL TESTED</u>	<u>OBSERVATION*</u>
MINIMAL GLUCOSE AGAR PLATES	NG
TOP AGAR	NG
DILUENT WATER	NG
NUTRIENT BROTH	NG
TEST COMPOUND (HIGHEST DOSE)	NG
S-9	NG

*G = Growth, NG = No Growth

TABLE 3: Mutagenicity Assay for 2-(E)-HYDROXYIMINOMETHYL-1-METHOXYMETHYL-3-METHYLIMIDAZOLIUM CHLORIDE (TP50)*

COMPOUND†	DOSE	TA98		TA100	
<u>WITHOUT S-9</u>					
NEG CONTROL	0.0 mg	17	± 5.9	119	± 16.7
MNNG	2.0 µg	-	-	1802	±305.5
MNNG	20.0 µg	-	-	-	-
TP50	5.0 mg	10	± 5.3	82	± 19.8
TP50	1.0 mg	10	± 4.4	58	± 14.6
TP50	0.2 mg	15	± 3.5	88	± 9.1
TP50	0.04 mg	12	± 4.4	101	± 21.1
TP50	0.008 mg	15	± 2.6	87	± 5.0
TP50	0.0016 mg	15	± 1.2	80	± 6.0
<u>WITH S-9</u>					
NEG CONTROL	0.0 mg	20	± 6.7	74	± 15.2
AA	2.0 µg	418	±132.1	575	± 28.2
AF	2.0 µg	353	± 64.4	137	± 9.8
BP	2.0 µg	240	± 43.5	164	± 19.1
TP50	5.0 mg	21	± 2.0	95	± 18.8
TP50	1.0 mg	14	± 3.5	65	± 3.2
TP50	0.2 mg	20	± 1.5	70	± 3.0
TP50	0.04 mg	23	± 4.5	72	± 6.7
TP50	0.008 mg	23	± 1.5	80	± 20.5
TP50	0.0016 mg	21	± 5.9	55	± 6.8

*Values represent the mean number of revertants/plate (± standard deviation)

†MNNG=N-methyl-N'-nitro-N-nitrosoguanidine, AA=2-aminoanthracene, AF=2-aminofluorene, BP=benzo[a]pyrene.

TABLE 3 (cont.): Mutagenicity Assay for 2-(E)-HYDROXYIMINOMETHYL-1-METHOXYMETHYL-3-METHYLIMIDAZOLIUM CHLORIDE (TP50) *

COMPOUND†	DOSE/PLATE	TA1535	TA1537	TA1538
WITHOUT S-9				
NEG CONTROL	0.0 mg	39 ± 6.0	6 ± 2.4	14 ± 2.5
MNNG	2.0 µg	-	-	-
MNNG	20.0 µg	1798 ± 255.1	-	-
TP50	5.0 mg	18 ± 4.9	3 ± 1.0	9 ± 2.3
TP50	1.0 mg	18 ± 3.2	3 ± 1.5	5 ± 4.0
TP50	0.2 mg	23 ± 7.5	4 ± 0.0	11 ± 1.2
TP50	0.04 mg	20 ± 6.0	6 ± 3.2	7 ± 4.9
TP50	0.008 mg	19 ± 4.7	5 ± 2.0	8 ± 1.2
TP50	0.0016 mg	20 ± 2.1	3 ± 4.0	12 ± 2.0
WITH S-9				
NEG CONTROL	0.0 mg	27 ± 17.1	6 ± 2.7	19 ± 6.6
AA	2.0 µg	-	164 ± 88.0	549 ± 54.5
AF	2.0 µg	-	-	320 ± 62.6
BP	2.0 µg	-	44 ± 13.1	102 ± 10.1
TP50	5.0 mg	10 ± 2.0	5 ± 1.0	12 ± 2.0
TP50	1.0 mg	8 ± 2.0	6 ± 3.2	10 ± 1.0
TP50	0.2 mg	11 ± 1.0	4 ± 2.5	12 ± 1.5
TP50	0.04 mg	11 ± 8.5	3 ± 1.2	17 ± 1.7
TP50	0.008 mg	14 ± 5.0	12 ± 5.9	9 ± 8.0
TP50	0.0016 mg	13 ± 5.1	9 ± 1.0	17 ± 8.7

*Values represent the mean number of revertants/plate (± standard deviation)

†MNNG=N-methyl-N'-nitro-N-nitrosoguanidine, AA=2- aminoanthracene, AF=2-aminofluorene, BP=benzo[a]pyrene.

CONCLUSION

2-(E)-HYDROXYIMINOMETHYL-1-METHOXYMETHYL-3-METHYLIMIDAZOLIUM CHLORIDE was evaluated for mutagenic potential in the Ames Test, in both the presence and the absence of metabolic activation, and did not induce a positive mutagenic response under conditions of this study.

REFERENCES

1. McCann J, Choi E, Yamasaki E, Ames BN. Detection of carcinogens as mutagens in the *Salmonella*/Mammalian Microsome Mutagenicity Test: Test of 300 chemicals. *Proc Nat Acad Sci, USA* 1975;72:5135-5139.
2. Ames *Salmonella*/Mammalian Microsome Mutagenesis Test. LAIR Standard Operating Procedure OP-STX-1, Presidio of San Francisco, California: Letterman Army Institute of Research, 29 August 1986.
3. Ames BN, McCann J, Yamasaki E. Methods for detection of carcinogens and mutagens with *Salmonella*/Mammalian Microsome Mutagenicity Test. *Mutat Res* 1975;31:347-364.
4. Vogel HJ, Bonner DM. Acetylornithinase of *E. coli*: Partial purification and some properties. *J Biol Chem* 1956;218:97-106.
5. Brusick D. Genetic toxicology. In: Hayes AW, ed. *Principles and methods of toxicology*. New York: Raven Press, 1982:223-272.

APPENDICES

APPENDIX A: Chemical Data	13
APPENDIX B: Individual Plate Scores	15

APPENDIX A: Chemical Data

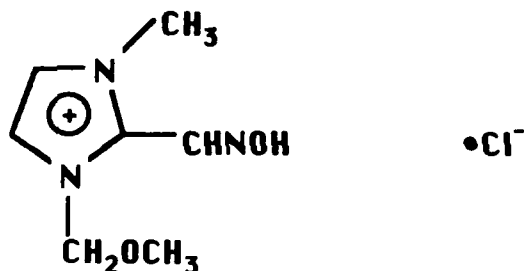
Chemical Name: 2-((hydroxyimino)methyl)-1-methoxymethyl)-3-methyl-1H-imidazolium chloride

Alternate Chemical Names: 2[(Hydroxyimino)methyl]-1-(methoxymethyl)-3-methylimidazolium chloride,
2-(E)-Hydroxyiminomethyl-1-methoxymethyl-3-methylimidazolium chloride

Chemical Abstracts Service Registry No: 91900-09-3

LAIR Code Number: TP50

Chemical Structure:



Molecular Formula: C₇H₁₂ClN₃O₂

Molecular Weight: 205.5

Source: Clifford D. Bedford, PhD
SRI International, Physical Sciences Division
Menlo Park, CA

SRI Reference Number: BHH-0001

APPENDIX A (cont.): Chemical Data

Analytical Data: Data supplied by SRI International included melting point, elemental analysis, and NMR and IR spectra.¹ Melting point: 168-172°C (dec). Elemental analysis calculated for $C_7H_{12}ClN_3O_2$: C, 40.88; H, 5.88; N, 20.43; Cl, 17.24. Found: C, 41.00; H, 6.01; N, 20.67; Cl, 17.02. ¹H NMR (60 MHz, d_6 -DMSO) δ 3.37 (s, 3H, OCH₃), 4.03 (s, 3H, NCH₃), 5.77 (s, 2H, NCH₂O), 8.08 (d, 1H, J=1.5 Hz, aryl), 8.17 (d, 1H, J=1.5 Hz, aryl), 8.62 (s, 1H, CHN), 13.45 (s, 1H, NOH). IR (KBr) 2700 (broad), 1590, 1525, 1420, 1395, 1320, 1300, 1270, 1240, 1205, 1120, 1060, 1005, 925, 875, 790 cm^{-1} . The IR spectrum obtained upon receipt of the compound confirmed the identity of the material.²

¹ Bedford CD. Notebook reference 5337-15. Stanford Research International, Physical Sciences Division, Menlo Park, CA.

² Wheeler CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #84-05-010.3, p18. Letterman Army Institute of Research, Presidio of San Francisco, CA.

APPENDIX B: Individual Plate Scores

2-(E)-HYDROXYIMINOMETHYL-1-METHOXYMETHYL-3-METHYLIMIDAZOLIUM CHLORIDE (TP50)

TOXICITY DETERMINATION WITH TA100

DOSE/PLATE	5.0 mg	1.0 mg	0.2 mg	0.04 mg
PLATE 1	71	101	118	126
PLATE 2	125	126	129	106
PLATE 3	127	109	120	131
background lawn	NL*	NL	NL	NL
DOSE/PLATE	0.008 mg	0.0016 mg	NEG CONTROL	
PLATE 1	111	125	114	
PLATE 2	133	128	123	
PLATE 3	137	112	125	
background lawn	NL	NL	NL	

* NL=Normal Lawn

APPENDIX B (cont.): Individual Plate Scores

2-(E)-HYDROXYIMINOMETHYL-1-METHOXYMETHYL-3-METHYLIMIDAZOLIUM CHLORIDE (TP50)

NEGATIVE CONTROL DATA

COMPOUND	DOSE/PLATE	TA98	TA100	TA1535	TA1537	TA1538
NEG CONTROL (START RUN)	0.0 mg	21	110	44	5	15
		17	129	39	3	13
		10	99	48	9	10
NEG CONTROL (END RUN)	0.0 mg	16	142	32	4	12
		12	130	36	7	15
		26	106	35	8	17
NEG CONTROL (START RUN)	0.0 mg	26	69	47	2	14
		25	64	30	6	12
		8	55	47	5	13
NEG CONTROL (END RUN)	0.0 mg	21	86	8	8	24
		19	74	16	10	25
		24	97	14	6	26

WITHOUT S-2WITH S-2

APPENDIX B (cont.): Individual Plate Scores

2-(E)-HYDROXYIMINOMETHYL-1-METHOXYMETHYL-3-METHYLIMIDAZOLIUM CHLORIDE (TP50)

POSITIVE CONTROL DATA

COMPOUND†	DOSE/PLATE	TA98	TA100	TA1535	TA1537	TA1538
AA	2.0 µg	294 403 557	555 562 607		266 114 113	585 486 575
AF	2.0 µg	280 402 377	129 148 134			367 344 249
BP	2.0 µg	261 190 269	170 180 143		38 59 35	93 113 101
MNNG	2.0 µg		1521 1757 2127			
MNNG	20.0 µg			2063 1778 1554		

†AA=2-aminoanthracene, AF=2-aminofluorene, BP=benzo[a]pyrene, MNNG=N-methyl-N'-nitro-N-nitrosoguanidine

2-(E)-HYDROXYIMINOMETHYL-1-METHOXYMETHYL-3-METHYLIMIDAZOLIUM CHLORIDE (TP50)

MUTAGENICITY DATA WITHOUT S-9

[illegible]

APPENDIX B (cont.): Individual Plate Scores

2-(E)-HYDROXYIMINOMETHYL-1-METHOXYMETHYL-3-METHYLIMIDAZOLIUM CHLORIDE (TP50)

MUTAGENICITY DATA WITH S-9

COMPOUND	DOSE/PLATE	TA98	TA100	TA1535	TA1537	TA1538
TP50	5 mg	23	104	10	5	12
		19	73	8	6	14
		21	107	12	4	10
TP50	1 mg	16	66	8	10	10
		16	67	6	4	9
		10	61	10	5	11
TP50	0.2 mg	20	73	10	2	12
		19	67	11	7	10
		22	70	12	4	13
TP50	0.04 mg	23	80	20	4	18
		19	68	3	2	18
		28	69	10	4	15
TP50	0.008 mg	25	91	15	8	10
		22	92	9	10	1
		23	56	19	19	17
TP50	0.0016 mg	14	47	19	8	12
		23	60	9	10	12
		25	57	12	9	27

OFFICIAL DISTRIBUTION LIST

Commander
US Army Medical Research
& Development Command
ATTN: SGRD-RMS/Mrs. Madigan
Fort Detrick, MD 21701-5012

Defense Technical Information Center
ATTN: DTIC/DDAB (2 copies)
Cameron Station
Alexandria, VA 22304-6145

Office of Under Secretary of Defense
Research and Engineering
ATTN: R&AT (E&LS), Room 3D129
The Pentagon
Washington, DC 20301-3080

DASG-AAFJML
Army/Air Force Joint Medical Library
Offices of the Surgeons General
5109 Leesburg Pike, Room 670
Falls Church, VA 22041-3258

HQ DA (DASG-ZXA)
WASH DC 20310-2300

Commandant
Academy of Health Sciences
US Army
ATTN: HSHA-CDM
Fort Sam Houston, TX 78234-6100

Uniformed Services University of
Health Sciences
Office of Grants Management
4301 Jones Bridge Road
Bethesda, MD 20814-4799

US Army Research Office
ATTN: Chemical and Biological
Sciences Division
PO Box 12211
Research Triangle Park, NC 27709-2211

Director
ATTN: SGRD-UWZ-L
Walter Reed Army Institute of Research
Washington, DC. 20307-5100

Commander
US Army Medical Research Institute
of Infectious Diseases
ATTN: SGRD-ULZ-A
Fort Detrick, MD 21701-5011

Commander
US Army Medical Bioengineering Research
and Development Laboratory
ATTN: SGRD-UBG-M
Fort Detrick, Bldg 568
Frederick, MD 21701-5010

Commander
US Army Medical Bioengineering
Research & Development Laboratory
ATTN: Library
Fort Detrick, Bldg 568
Frederick, MD 21701-5010

Commander
US Army Research Institute
of Environmental Medicine
ATTN: SGRD-UE-RSA
Kansas Street
Natick, MA 01760-5007

Commander
US Army Research Institute of
Surgical Research
Fort Sam Houston, TX 78234-6200

Commander
US Army Research Institute of
Chemical Defense
ATTN: SGRD-UV-AJ
Aberdeen Proving Ground, MD 21010-5425

Commander
US Army Aeromedical Research
Laboratory
Fort Rucker, AL 36362-5000

AIR FORCE Office of Scientific
Research (NL)
Building 410, Room A217
Bolling Air Force Base, DC 20332-6448

USAF School of Aerospace Medicine
Document Section
USAFSAM/TSKD
Brooks Air Force Base, TX 78235-5301

Head, Biological Sciences Division
OFFICE OF NAVAL RESEARCH
800 North Quincy Street
Arlington, VA 22217-5000